Cancer Causes and Control **13**: 617–623, 2002.

Effects of alpha-tocopherol and beta-carotene supplementation on gastric cancer incidence in male smokers (ATBC Study, Finland)

Nea Malila[1,*], Philip R. Taylor[2], Mikko J. Virtanen[1], Pasi Korhonen[1], Jussi K. Huttunen[1], Demetrius Albanes[2] & Jarmo Virtamo[1]
[1]*National Public Health Institute, Helsinki, Finland;* [2]*National Cancer Institute, Bethesda, MD, USA*

Received 29 November 2001; accepted in revised form 28 March 2002

Key words: beta-carotene, chemoprevention, randomized controlled trials, stomach neoplasms, vitamin E.

Abstract

Objectives: This study investigated the effects of alpha-tocopherol and beta-carotene supplementation on the incidence of gastric cancer.
Methods: A total of 29,133 male smokers, aged 50–69 years, participated in a placebo-controlled prevention trial, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study in southwestern Finland between 1985 and 1993. The men were randomly assigned to receive alpha-tocopherol (50 mg/day) or beta-carotene (20 mg/day) supplementation in a 2×2 factorial design. We identified 126 gastric cancer cases during the median follow-up of six years. Of these, 122 were adenocarcinomas: 75 of intestinal type, 30 of diffuse type, and 17 of mixed type.
Results: There was no significant effect for either supplementation on the overall incidence of gastric cancer: relative risk (RR) 1.21, 95% confidence interval (CI) 0.85–1.74 for alpha-tocopherol, and RR 1.26, 95% CI 0.88–1.80 for beta-carotene. Subgroup analyses by histologic type suggested an increased risk for beta-carotene on intestinal type cancers, RR 1.59, 95% CI 0.99–2.56. There were no differences across anatomic locations (cardia/noncardia) in the effects of alpha-tocopherol or beta-carotene supplementation.
Conclusions: Our study found no overall preventive effect of long-term supplementation with alpha-tocopherol or beta-carotene on gastric cancer in middle-aged male smokers.

Introduction

Gastric cancer remains the second most common cancer and cause of cancer death throughout the world [1], although its incidence has been declining in the western industrialized world. The areas with the highest incidence rates are in eastern Asia, South America, and eastern Europe. Low rates are found in North America, northern Europe, and most of Africa [2]. In the 1950s gastric cancer was the leading cancer of both men and women in Finland, but since then a steady decrease in the risk has taken place, and in 1999 gastric cancer was the fifth most common incident cancer among Finnish men and the ninth among women (http://www.cancer-registry.fi/eng/statistics.htm) [3]. The age-adjusted relative 5-year survival rate remains poor, however, at approximately 20% [2].

Most stomach cancers are adenocarcinomas which can be further divided into two histologic subcategories: an intestinal type with cohesive neoplastic cells forming glandlike tubular structures, and a diffuse type in which individual cells infiltrate and thicken the stomach wall without any discrete mass [4]. The intestinal type of adenocarcinoma predominates in high-risk areas, whereas the incidence of the diffuse type is similar in most populations throughout the world [2].

The etiology of gastric carcinoma is multifactorial and most commonly develops after a long period of atrophic gastritis [5]. The incidence of gastric cancer increases rapidly after the age of 40 and the highest incidence is found in the oldest age groups, in both men and women [5]. However, the intestinal type rises faster with age than the diffuse type and is more frequent in men than in women. *Helicobacter pylori* infection has been

* Address correspondence to: Nea Malila, Finnish Cancer Registry, Liisankatu 21 B, 00170 Helsinki, Finland. Ph.: +358 9 135 33 271; Fax: +358 9 135 5378; E-mail: Nea.Malila@cancer.fi

recognized as a significant risk factor for gastric cancer [6]. *H. pylori* has been shown to induce the phenotypic changes (including mucosal atrophy, intestinal metaplasia, and dysplasia) leading up to the development of adenocarcinoma. The association between *H. pylori* infection and gastric cancer has not been restricted to either histologic type of adenocarcinomas or to any specific subsite within the stomach [6].

The intestinal type of gastric cancer has also been related to diet. A high intake of fresh fruit and vegetables is associated with lower risk, with ascorbic acid, carotenoids, folates, and tocopherols suggested as the protective agents. Foods associated with high risk include smoked fish or meats, pickled vegetables, and excessive salt intake [1]. Studies on alcohol and tobacco have yielded inconsistent associations [5].

The aim of the present study was to investigate the effect of supplemental alpha-tocopherol and beta-carotene on gastric cancer incidence in older male smokers participating in a controlled cancer prevention trial. In addition, we studied the effect of these supplements on different histologic subtypes of gastric cancer, as well as the effect on anatomic subsites (cardia or noncardia).

Materials and methods

This study was conducted as part of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, a randomized, double-blind, placebo-controlled, two-by-two factorial primary prevention trial that tested the effect of alpha-tocopherol (50 mg/day) and beta-carotene (20 mg/day) supplementation on lung cancer incidence [7]. Participants in the trial were male smokers, aged 50–69 years at study entry, who lived in southwestern Finland. In all, 29,133 men were recruited into the trial between 1985 and 1988, and the trial ended on 30 April 1993 after 5–8 years of active intervention (median 6.1 years). At baseline a detailed dietary history questionnaire was obtained, weight and height were measured, and a fasting serum sample was taken. Study eligibility was assessed before randomization; subjects with prior cancer or serious illness, or those using vitamin E, A, or beta-carotene supplements, were excluded.

Endpoint assessment

The cancer cases were identified through the Finnish Cancer Registry (FCR), and death certificates. The FCR collects data on all cancer cases in Finland and the coverage and accuracy of their data have been evaluated: almost 99% of all cancers nationwide can be found in files of the FCR [8].

During the trial 130 gastric cancers (ICD9 code 151) were identified and confirmed from the study population. The medical records were collected and reviewed by two oncologists and the histology was checked and determined according to the Lauren classification [4] by two pathologists. Four carcinoid tumors were excluded, leaving 126 cases for analysis. There were 122 cases with histology being adenocarcinoma, of which 75 cases were of the intestinal type, 30 cases were of the diffuse type, and 17 cases were of mixed type. One case was unspecified carcinoma, and three cases were based on clinical diagnosis with no histology available. Gastric cancers were also coded according to the anatomic subsite within the stomach: 28 cardia cancers and 98 noncardia cancers. Nearly all cardia cancers were of the intestinal type, with only two cases being of the mixed type, and one case of diffuse type adenocarcinoma. Of the noncardia cancers, 50 were intestinal type adenocarcinomas, 29 were of the diffuse type, and 15 of the mixed type. The three clinical cases and the one case of unspecified carcinoma were all noncardia cancers.

Staging was based on the criteria of the American Joint Committee on Cancer [9]. Of the 126 cases, 40 (32%) were stage I, 13 (10%) were stage II, 27 (21%) were stage III, and 46 (37%) were stage IV when diagnosed.

Statistical analysis

Analyses estimating the effects of trial supplementation on gastric cancer incidence were based on the intention-to-treat principle, and relative risks with their 95% confidence intervals were calculated using the proportional hazards model [10]. Interactions between baseline characteristics and study supplementation were tested with the same model.

Follow-up time accumulated from randomization to date of diagnosis of gastric cancer, death, or 30 April 1993 whichever occurred first. We tested the interaction between study supplementation and continuous background variables as quartiles using a categorical indicator variable for each quartile. These included baseline intake and serum concentration of alpha-tocopherol and beta-carotene; age; body mass index; intake of alcohol, vitamin C, sodium, nitrates and nitrites; years as a smoker; and number of cigarettes smoked per day. We also studied the interaction between trial supplementation and recruitment area, history of gastric ulcer at baseline, and baseline serum pepsinogen I level. The interaction between study supplements and the presence of *H. pylori* infection was studied in a case–cohort setting with nearly all cases (118 out of 126 had serology available) and 285 noncases. The noncases were a

random sample of study subjects free of gastric cancer by 30 April 1993. Anti-*H. pylori* IgA and IgG were determined separately by enzyme immunoassay [11]. The sensitivity and specificity of the IgA test were 73.1% and 95.1% and for the IgG test, 93.7% and 93.9%. *H. pylori* status was considered negative if both IgA and IgG antibodies were negative in the baseline serum (cut-off points 1:70 for IgA and 1:700 for IgG antibodies), and positive if either was positive.

The associations between baseline dietary and serum alpha-tocopherol and beta-carotene (modeled as quartiles) and the incidence of gastric cancer were calculated by Cox models adjusting for study supplementation (alpha-tocopherol *versus* no alpha-tocopherol or beta-carotene *versus* no beta-carotene), and in serum analyses additionally for age, number of cigarettes smoked per day, and serum cholesterol concentration.

Results

Table 1 shows the numbers of gastric cancer cases by anatomic subsite and specific histology in the four supplementation groups. The incidence rates were higher in all active agent supplementation groups compared to the placebo group, but the relative risk estimates did not differ significantly from one (Table 2). When comparing those supplemented with alpha-tocopherol to those not supplemented with alpha-tocopherol the relative risk (RR) for gastric cancer was 1.21 (95% confidence interval, CI, 0.85–1.74), and the respective RR for beta-carotene was 1.26 (95% CI 0.88–1.80) (Table 3). The Kaplan–Meier cumulative incidence for alpha-tocopherol and beta-carotene supplementation indicated no clear difference between the curves (Figure 1).

Subgroup analyses of cardia cancers showed no effect for alpha-tocopherol supplementation but suggested excess incidence for beta-carotene supplementation (RR 1.81, 95% CI 0.82–3.98) (Table 3). For noncardia cancers there was no effect for either supplement. When the histologic subtype was taken into consideration, beta-carotene supplementation showed a nearly significant increase in risk for adenocarcinomas of the intestinal type (RR 1.59, 95% CI 0.99–2.56).

Alpha-tocopherol supplementation increased slightly, though not significantly, the risk of advanced gastric cancer (stages III and IV) (RR 1.44, 95% CI 0.89–2.31), whereas it showed no effect on localized disease (stages I and II) (RR 0.96, 95% CI 0.56–1.67). The beta-carotene effect was similar in both local and advanced cases (data not shown).

Statistically significant interactions between study supplementation and other characteristics were observed for vitamin E intake and *H. pylori* status, and a suggestive trend was observed with increasing age for both agents (Table 4). Other variables examined, including baseline serum concentration of alpha-tocopherol

Table 1. Incident gastric cancer cases in the ATBC Study (intervention period) according to supplementation group

Gastric cancers	No. of cases	Placebo	Alpha-tocopherol	Alpha-tocopherol and beta-carotene	Beta-carotene
All cases	126	24	32	37	33
Subsite					
Cardia	28	4	6	8	10
Noncardia	98	20	26	29	23
Adenocarcinoma subtype[a]	122				
Intestinal	75	13	16	21	25
Diffuse	30	7	8	9	6
Mixed	17	3	7	5	2

[a] Three clinical cases and one unspecified carcinoma not included.

Table 2. Incidence rates, relative risks, and 95% confidence intervals (CI) for gastric cancer in the four ATBC Study supplementation groups

	Placebo	Alpha-tocopherol	Alpha-tocopherol and beta-carotene	Beta-carotene
Participants	7,287	7,286	7,278	7,282
Gastric cancers	24	32	37	33
Person-years	42,530	42,439	42,300	42,359
Rate/10,000 person-years	5.64	7.54	8.75	7.79
Relative risk (95% CI)	1.00	1.34 (0.78–2.29)	1.55 (0.92–2.62)	1.38 (0.81–2.36)

Table 3. Relative risks (RR) and 95% confidence intervals (CI) of gastric cancer for the supplementation groups compared to the groups not receiving the supplements

Gastric cancers	No. of cases	Alpha-tocopherol *vs* no alpha-tocopherol		Beta-carotene *vs* no beta-carotene	
		RR	95% CI	RR	95% CI
All cases	126	1.21	0.85–1.74	1.26	0.88–1.80
Subsite					
Cardia	28	1.00	0.47–2.13	1.81	0.82–3.98
Noncardia	98	1.27	0.85–1.89	1.13	0.76–1.68
Histologic type					
Intestinal	75	0.98	0.61–1.55	1.59	0.99–2.56
Diffuse	30	1.53	0.88–2.67	1.00	0.48–2.08

or beta-carotene; age; body mass index; intake of beta-carotene, alcohol, vitamin C, sodium, nitrates, and nitrites; years as a smoker; number of cigarettes per day; recruitment area; history of gastric ulcer; and baseline serum pepsinogen I level did not modify the effect of either supplement.

Baseline intakes and serum concentrations of alpha-tocopherol and beta-carotene were not materially associated with subsequent gastric cancer (data not shown).

Discussion

We found no overall effect for alpha-tocopherol or beta-carotene supplementation on the incidence of gastric cancer in older male smokers. Only three primary cancer prevention trials have reported findings concerning effects of supplements in relation to gastric cancer outcomes. Our null result for beta-carotene was similar to that of the Physicians' Health Study, where 19 gastric cancer cases were identified in the beta-carotene group and 21 in the placebo group [12]. The number of cases in that trial was substantially smaller, however, than in the ATBC Study. The Nutrition Intervention Trial in the general population from Linxian, China, found a 16% reduction in gastric cancer incidence and a 21% reduction in gastric cancer mortality among participants who received the combination of beta-carotene, vitamin E, and selenium [13]. Over three-fourths of the cases were from the cardia, but results for both cardia and non-cardia were similar. The specificity of these findings is complicated due to the use of an antioxidant cocktail, and generalizability to well-nourished western populations is compromised since this population in China was marginally deficient in a number of nutrients. Another trial in Linxian was conducted in persons with esophageal dysplasia who were randomly assigned to receive daily supplementation with 14 vitamins and 12 minerals (including 15 mg beta-carotene and 60 IU alpha-tocopherol) or placebo [14]. No effect was observed during this six-year intervention on overall gastric cancer incidence or mortality; however, among the small number of incident gastric cancers in the noncardia ($n = 18$), the relative risk was significantly higher in the supplemented group. A factorial chemoprevention trial in a high-risk population in Colombia found an increased rate of precancerous gastric lesion regression among participants supplemented with ascorbic acid or beta-carotene, or treated with triple therapy for *H. pylori* infection compared to the placebo group [15]. However, care is suggested in interpreting these findings because almost all of the benefit appeared to be due to the especially low regression rate in the placebo group [16].

Epidemiologic studies have implicated a role for consumption of fruits and vegetables and intake of antioxidants, such as vitamins E and C and carotenoids, in gastric carcinogenesis. Why, then, do the results of controlled trials differ from these findings? It may be that other compounds found in fruits and vegetables are responsible for the inverse associations in epidemiologic studies. It is also possible that antioxidants have a favorable effect in an early phase of carcinogenesis and studies of dietary intake usually reflect longer-term exposures (even lifelong) than the six years that our trial covered.

In histologic subtype analyses there was a suggestion of increased risk of intestinal adenocarcinomas in the beta-carotene group that was not formally significant. This finding should be interpreted cautiously, however, since it was not based on an *a-priori* hypothesis, may have occurred by chance due to multiple comparisons, and there we are aware of no biological mechanism through which beta-carotene could exert such an influence.

Previous epidemiologic studies reporting histologic subtypes of gastric adenocarcinoma are sparse. Case–control studies in Sweden, Italy, and the US have reported associations between dietary beta-carotene and

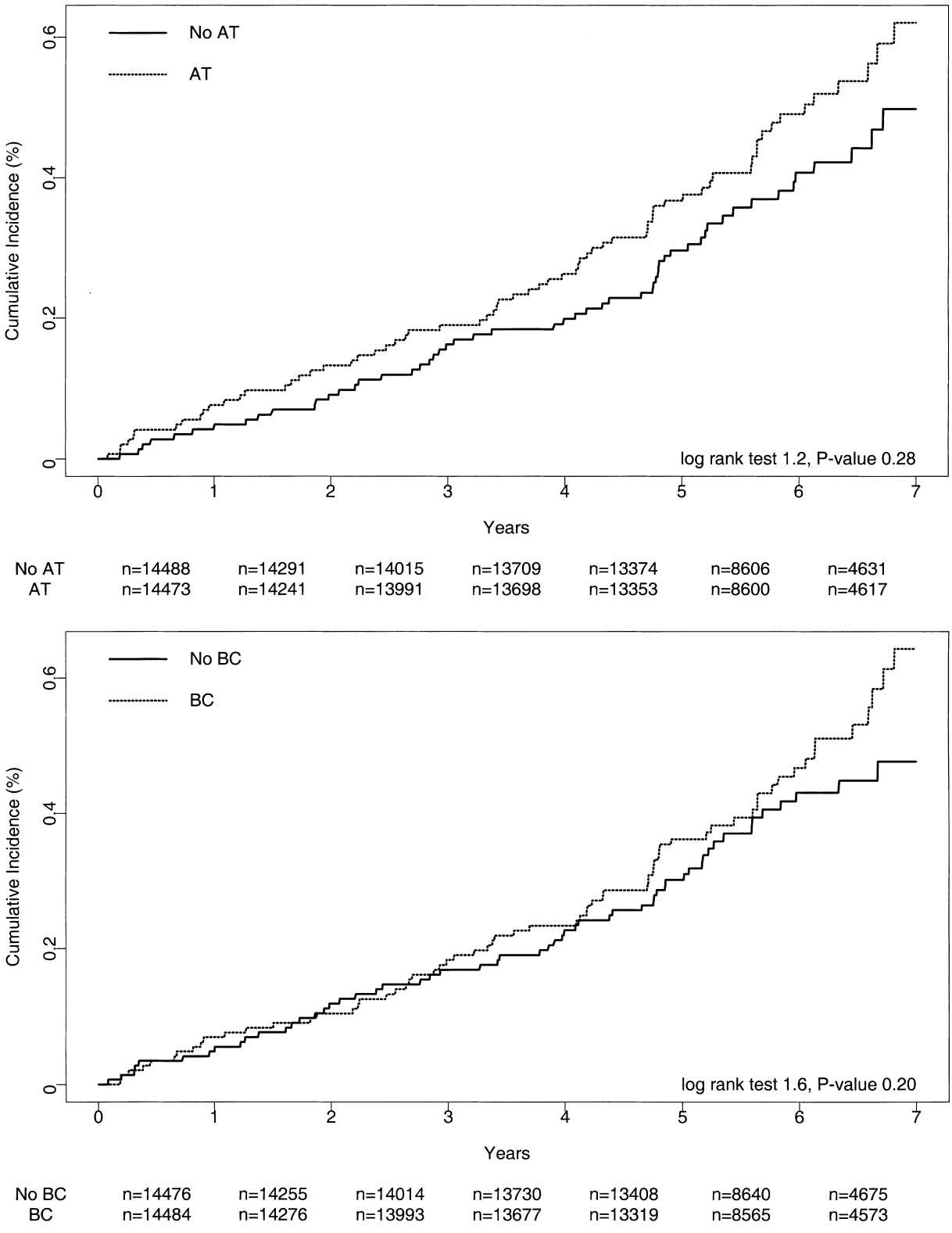

Fig. 1. Kaplan–Meier cumulative incidence curves for those supplemented with alpha-tocopherol (AT) (top panel) and those not supplemented with alpha-tocopherol (no AT) and respective curves for beta-carotene (BC) (lower panel) and no beta-carotene (no BC) supplementation. Number of cases at risk by year below the figure for both supplementation arms separately.

Table 4. Relative risks (RR) and their 95% confidence intervals (CI) for selected interactions between ATBC Study supplementation and baseline characteristics

	No. of cases	Alpha-tocopherol *vs* no alpha-tocopherol RR (95% CI)	Beta-carotene *vs* no beta-carotene RR (95% CI)
Age (years)			
50–55	21	0.76 (0.31–1.38)	0.52 (0.21–1.31)
>55–60	48	1.10 (0.62–1.95)	1.17 (0.66–2.09)
>60–65	37	1.26 (0.65–2.45)	1.62 (0.82–3.18)
>65–69	20	2.42 (0.91–6.41)	2.28 (0.86–6.05)
p for interaction		0.33	0.11
Vitamin E intake (mg/day)			
<8.2	32	1.02 (0.50–2.07)	3.12 (1.38–7.06)
8.2–10.7	25	0.56 (0.24–1.29)	0.69 (0.31–1.56)
10.8–14.5	27	1.22 (0.56–2.64)	0.77 (0.36–1.67)
>14.5	26	1.94 (0.85–4.42)	1.36 (0.62–3.01)
p for interaction		0.18	0.02
H. pylori serology			
Negative	17	3.47 (1.03–11.67)	0.58 (0.20–1.72)
Positive[a]	101	1.00 (0.61–1.65)	1.30 (0.79–2.15)
p for interaction		0.03	0.13

[a] Sixty-nine percent of the noncases had positive *H. pylori* serology.

gastric cancer separating intestinal cancers and diffuse cancers [17–19]. In these studies dietary beta-carotene was significantly inversely related to adenocarcinomas of the intestinal type. A prospective case–cohort study from the Netherlands observed a positive association between beta-carotene intake and gastric carcinoma, but subgroup analyses among different histologic subtypes were not available from this study [20]. Additional studies suggest a role for fruit and vegetable consumption *per se* [21].

In conclusion, we did not find any preventive effects of alpha-tocopherol or beta-carotene supplementation, taken for an average of 6.1 years, on the overall incidence of gastric cancer.

Acknowledgements

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study was supported by a contract with the US National Cancer Institute (N01-CN-45165). The work was performed at the Department of Epidemiology and Health Promotion, National Public Health Institute, Helsinki, Finland.

References

1. Cannon G, ed. (1997) *Food, Nutrition and the Prevention of Cancer: a Global Perspective*. Washington, DC: American Institute for Cancer Research.
2. Fuchs CS, Mayer RJ (1995) Gastric carcinoma. *N Engl J Med* **333**: 32–41.
3. Cancer Statistics of the National Research and Development Centre for Welfare and Health (2000) *Cancer Incidence in Finland 1996 and 1997*. Helsinki: Cancer Society of Finland.
4. Lauren P (1965) The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. *Acta Pathol Microbiol Scand* **64**: 31–49.
5. Fenoglio-Preiser C, Carneoiro F, Correa P, *et al*. (2000) Gastric carcinoma. In: Hamilton SR, Aaltonen LA, eds. *World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Digestive System*. Lyon: IARC Press, pp. 37–66.
6. Parsonnet J, Friedman GD, Vandersteen DP, *et al*. (1991) *Helicobacter pylori* infection and the risk of gastric carcinoma. *N Engl J Med* **325**: 1127–1131.
7. ATBC Cancer Prevention Study Group (1994) The alpha-tocopherol, beta-carotene lung cancer prevention study: design, methods, participant characteristics, and compliance. *Ann Epidemiol* **4**: 1–10.
8. Teppo L, Pukkala E, Lehtonen M (1994) Data-quality and quality control of a population-based cancer registry. *Acta Oncol* **33**: 365–369.
9. Beahrs OH, Henson DE, Hutter RVP, Kennedy BJ, eds. (1992) *Manual for Staging of Cancer*. Philadelphia: J. B. Lippincott.
10. Cox DR, Oakes D (1984) *Analysis of Survival Data*. London: Chapman & Hall.
11. Aromaa A, Kosunen TU, Knekt P, *et al*. (1996) Circulating anti-*Helicobacter pylori* immunoglobulin A antibodies and low serum pepsinogen I level are associated with increased risk of gastric cancer. *Am J Epidemiol* **144**: 142–149.
12. Hennekens CH, Buring JE, Manson JE, *et al*. (1996) Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *N Engl J Med* **334**: 1145–1149.
13. Blot WJ, Li J-Y, Taylor PR, *et al*. (1993) Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/

mineral combinations, cancer incidence, and disease-specific mortality in the general population. *J Natl Cancer Inst* **85**: 1483–1492.
14. Li J-Y, Taylor PR, Li B, *et al*. (1993) Nutrition intervention trials in Linxian, China: multiple vitamin/mineral supplementation, cancer incidence, and disease-specific mortality among adults with esophageal dysplasia. *J Natl Cancer Inst* **85**: 1492–1498.
15. Correa P, Fontham ETH, Bravo JC, *et al*. (2000) Chemoprevention of gastric dysplasia: randomized trial of antioxidant supplements and anti-*Helicobacter pylori* therapy. *J Natl Cancer Inst* **92**: 1881–1888.
16. Blot WJ (2000) Preventing cancer by disrupting progression of precancerous lesions. *J Natl Cancer Inst* **92**: 1868–1869.
17. Ekström AM, Serafini M, Nyren O, Hansson L-E, Ye W, Wolk A (2000) Dietary antioxidant intake and the risk of cardia cancer and noncardia cancer of the intestinal and diffuse types: a population-based case–control study in Sweden. *Int J Cancer* **87**: 133–140.
18. Buiatti E, Palli D, Bianchi S, *et al*. (1991) A case–control study of gastric cancer and diet in Italy. III. Risk patterns by histologic type. *Int J Cancer* **48**: 369–374.
19. Harrison LE, Zhang Z-F, Karpeh MS, Sun M, Kurtz RC (1997) The role of dietary factors in the intestinal and diffuse histologic subtypes of gastric adenocarcinoma. *Cancer* **80**: 1021–1028.
20. Botterweck AAM, van den Brandt PA, Goldbohm RA (2000) Vitamins, carotenoids, dietary fiber, and the risk of gastric carcinoma. *Cancer* **88**: 737–748.
21. LaVecchia C, Franceschi S (2000) Nutrition and gastric cancer. *Can J Gastroenterol* **14** (Suppl D): 51D–54D.